JOURNAL OF BACTERIOLOGY, May 1988, p. 2056–2062
0021-9193/88/052056-07$02.00/0

Vol. 170, No. 5

The *lit* Gene Product Which Blocks Bacteriophage T4 Late Gene Expression Is a Membrane Protein Encoded by a Cryptic DNA Element, e14†

CHENG KAO AND LARRY SNYDER*

Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824-1101

Received 20 August 1987/Accepted 5 February 1988

***Escherichia coli lit*(Con) mutations cause a severe inhibition of gene expression late in infection by bacteriophage T4 owing to the overproduction of one, and possibly two, proteins (C. Kao, E. Gumbs, and L. Snyder, J. Bacteriol. 169:1232–1238, 1987). One or both of these proteins interact, either directly or indirectly, with a short sequence about one-quarter of the way into the major capsid protein gene of T4, and the inhibition occurs when this late gene of the virus is expressed. In this report we show that *lit*(Con) mutations are up-promoter mutations in the cryptic DNA element e14 and that only one of the proteins, gp*lit*, of about 34 kilodaltons, is required for the inhibition. We have sequenced the *lit* gene and the surrounding regions. From the sequence, and from cell fractionation studies, we conclude that gp*lit* is an inner membrane protein. Since the assembly of T4 heads is thought to occur on the inner face of the inner membrane, we propose that gp*lit* interferes with a normal regulation which coordinates the synthesis of proteins and the assembly of T4 heads.**

The gene expression of bacteriophage T4 requires both host and bacteriophage gene products. In an effort to identify *Escherichia coli* genes which can affect T4 gene expression, we have identified a type of *E. coli* mutation, *lit*(Con), so named because they cause a late inhibition of the gene expression of bacteriophage T4 (6, 9). The *lit*(Con) *E. coli* mutations all map at 25 min between the *purB* and *fadR* loci on the K-12 genetic map. We reported (12) that *lit*(Con) mutations cause the overproduction of at least one and possibly two proteins, of approximately 35 and 18 kilodaltons (kDa). One or both of these proteins were hypothesized to inhibit T4 gene expression and thereby prevent the production of progeny phage (12). The region of the *E. coli* chromosome harboring *lit*(Con) mutations was shown to be common to most, but not all, *E. coli* K-12 strains and missing in B strains. We hypothesized that *lit*(Con) mutations may be in a transposon or cryptic prophage.

T4 mutants that can multiply on *E. coli* with *lit*(Con) mutations have point mutations within a 40-nucleotide stretch of the major capsid gene of T4, gene 23 (7). These phage mutations are called *gol*, since they grow on *lit*(Con). Apparently, *gol* mutations alter a short sequence within gene 23 so that it no longer interacts with gp*lit*. However, only a very small part of gene 23 is required for the inhibition of gene expression, and plasmid clones containing only 75 base pairs of gene 23 will not transform *E. coli* with a *lit*(Con) mutation (7; K. Bergsland and L. R. Snyder, submitted for publication). The nascent polypeptide is at least partially responsible, since the region is not active if translated out of frame. We presented evidence that the presence of the Lit protein(s) triggers a termination in the *gol* region and this termination then triggers a general inhibition of gene expression (Bergsland and Snyder, submitted). In this paper we report the sequence of the region of *lit*(Con) mutations and the results of in vitro deletion experiments which show that only the protein of about 35 kDa is required for the phenotypes. We also present evidence that *lit*(Con) mutations are up-promoter mutations in the cryptic DNA element e14 and that the e14-encoded protein is localized in the inner membrane.

* Corresponding author.

† Journal article 12515 from the Michigan Agricultural Experimental Station.

MATERIALS AND METHODS

Bacteria, phages, and plasmid constructs. Table 1 summarizes the relevant features of the plasmids and *E. coli* strains used in our experiments. The phenotype of plasmids containing subclones of the region of *lit*(Con) mutations was determined by spotting 10^5 and 10^2 wild-type T4 phages and T4 phages with a *gol* mutation onto a lawn of *E. coli* containing the plasmid. P1 transduction was done by the method of Miller (20). JM101 *lit-6* was made by transducing the *lit-6* mutation from a donor carrying a Tn*10* in *fadR*, which is close to *lit*(Con) mutations, into the e14-containing JM101, selecting for tetracycline resistance. To make JM101 lit^0, the strain was then cured of the tetracycline resistance by fusaric acid selection (16) and transduced with P1 phage grown in cells cured of e14 but carrying the Tn*10* in *fadR*. The tetracycline-resistant transductants were scored for loss of the Lit(Con) phenotype.

Media and culture conditions. The *E. coli* strains were usually grown in either TB (0.5% NaCl, 1% tryptone, 1% Casamino Acids [Difco Laboratories] 20 μg of thiamine per ml) or LB (1% tryptone, 1% NaCl, 0.5% yeast extract). In the maxicell experiment, the cells were grown in M9 medium supplemented with 0.4% Casamino Acids. Top agar and plates were made with TB containing 0.7% and 1.5% agar, respectively. When appropriate, antibiotics were used in the following concentrations: ampicillin, 25 μg/ml; chloramphenicol, 20 μg/ml; and tetracycline, 10 μg/ml.

Nucleic acid purification. All plasmids used in subcloning were extracted by an alkaline lysis protocol (17) and purified on CsCl step gradients. RNAs were purified by the method of Aiba et al. (1). The purified RNAs were kept at −70°C after 2 volumes of ethanol had been added.

***Bal* 31 deletions and plasmid constructions.** Before *Bal* 31 deletions were made, the 2.3-kilobase-pair (kb) *Eco*RV insert from pAE1 that contains the wild-type *lit* region was

TABLE 1. Bacterial and phage strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference
E. coli strains		
JM101	F′ *lit*$^+$	29
JM101 *lit-6*	F′, transduced to *lit*(Con)	This work
JM101 *lit*^{0}	F′, transduced to *lit*^{0}	This work
1046	*hsdR recA*	Our laboratory
Bacteriophage		
T4 *gol*6B	T4 with spontaneous *gol* mutation	6
Plasmids		
pBR328	Cm^r Tet^r Amp^r; no *Bgl*II or *Xba*I sites; *Eco*RV site in *tet* gene	Boehringer Mannheim Biochemicals
pAE1	2.3-kb *Eco*RV subclone from AB2495	11
pM6E1	2.3-kb *Eco*RV subclone from MPH6	11
pM7E	2.3-kb *Eco*RV subclone from MPH7	This work
pM21E	2.3-kb *Eco*RV subclone from MPH21	This work
pABglII+4	pAE1 with filled-in *Bgl*II site	This work
pAXbaI+4	pAE1 with filled-in *Xba*I site	This work
pUAE1	2.3-kb *Eco*RV insert of pAE1 recloned into *Hin*cII site of pUC13	This work
pURI3	pUAE1 with *Bal* 31 deletion extending to 61 base pairs upstream of coding region of ORF1	This work
pURI20	pUAE1 with *Bal* 31 deletion extending 29 nucleotides into coding region of ORF1	This work
pURI3P	Subclone from deletion endpoint of RI3 to *Pvu*I site in ORF2 in pUC13	This work
pAG2	*Hin*dIII subclone of e14	C. Hill
pHB101	*Hin*dIII subclone of e14	C. Hill

recloned into the *Hin*cII site in the polylinker of pUC13 to generate pUAE1. The plasmid was then linearized with either *Eco*RI or *Pst*I, upstream or downstream of the insert, respectively, phenol-chloroform extracted, and ethanol precipitated. After treatment with *Bal* 31 (International Biotechnologies, Inc. [IBI], slow form) for an empirically determined length of time to give the desired extent of deletion, the reaction was stopped by phenol-chloroform extraction and ethanol precipitation. The plasmid was then digested to release an insert with one blunt end and one protruding end, electroeluted into DEAE-paper (Schleicher & Schuell, Inc.), recloned into pUC vectors, and transformed into *E. coli* JM101. The transformation protocol of Cohen et al. (8) was followed, except that the cells were washed with 100 mM $CaCl_2$–10 mM Tris hydrochloride (pH 8.0)–10 mM $MgCl_2$ before being resuspended in the same buffer.

pABglII+4 and pAXbaI+4 were constructed by linearizing pM6E1 at the unique *Bgl*II or *Xba*I site and then filling in the protruding 4-base-pair overhang with Klenow Fragment and all four deoxynucleoside triphosphates (Boehringer Mannheim Biochemicals). The blunt-ended plasmids were religated and transformed into JM101, selecting for chloramphenicol resistance. The plasmids in the transformants were screened for loss of the restriction sites.

Restriction enzymes and T4 DNA ligase were purchased from either IBI or Boehringer Mannheim and were used with supplied buffers.

Southern hybridization. A 1-μg portion of each DNA sample was digested with restriction enzyme and electrophoresed in a 0.9% agarose gel buffered with 0.089 M Tris, 0.089 M boric acid, and 2.8 mM EDTA (pH 8.3). The gel-fractionated DNAs were then transferred onto nitrocellulose filters (New England Nuclear Corp.) by Southern transfer (25) and probed with [α-^{32}P]dATP (IBI)-labeled nick-translated DNA (kit from Amersham Corp.), of at least 7 × 10^6 cpm/μg. Hybridization took place at 42°C in 10 volume of 50% formamide–5× Denhardt solution (1% Ficoll, 1% polyvinylpyrrolidone, 1% bovine serum albumin)–6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–100 μg of sheared and heat-denatured salmon sperm DNA per ml. The filter was washed for 15 min each with 2× SSC–1% sodium dodecyl sulfate at room temperature, 42°C, and 65°C in succession with one change of wash buffer at each temperature.

The recipes for the RNA electrophoresis in formaldehyde gels of 2% agarose and Northern (RNA) hybridization were described by Stuart et al. (26). The probe was the *Bgl*II-*Hin*dIII restriction fragment internal to the *lit* gene nick-translated to 5 × 10^6 cpm/μg of DNA. The hybridization took place for 14 h at 42°C. The filter was washed with 2× SSC–0.1% sodium dodecyl sulfate for 20 min each at 42 and 60°C with one change of buffer at each temperature and for 20 min with 1× SSC–0.1% sodium dodecyl sulfate at 60°C. The RNA markers were from Bethesda Research Laboratories.

DNA sequencing. The sequence of the wild-type *lit* region was determined by following the dideoxy protocol (23). The endpoints of the *Bal* 31 deletions 5′ of the *lit* gene were determined by Maxam-Gilbert sequencing (18) of DNAs singly labeled at the *Bgl*II site by Klenow fragment. The base pair changes of three independent *lit*(Con) mutations from the mutants MPH6, MPH7, and MPH21 (9) were determined by dideoxy sequencing, and the mutational changes in MPH7 and MPH21 were confirmed by Maxam-Gilbert sequencing with fragments labeled at the *Bgl*II site as above.

Maxicells and membrane fractionations. Maxicell labeling of plasmid-encoded proteins was done essentially by following the protocol of Sancar et al. (22) with the *recA* strain 1046 (Table 1). The UV radiation dose was empirically adjusted to minimize background labeling due to chromosomal transcription, and cycloserine was added to 200 μg/ml 1 and 4 h after irradiation. The labeling medium consisted of M9 growth medium supplemented with 50 μg of thiamine per ml

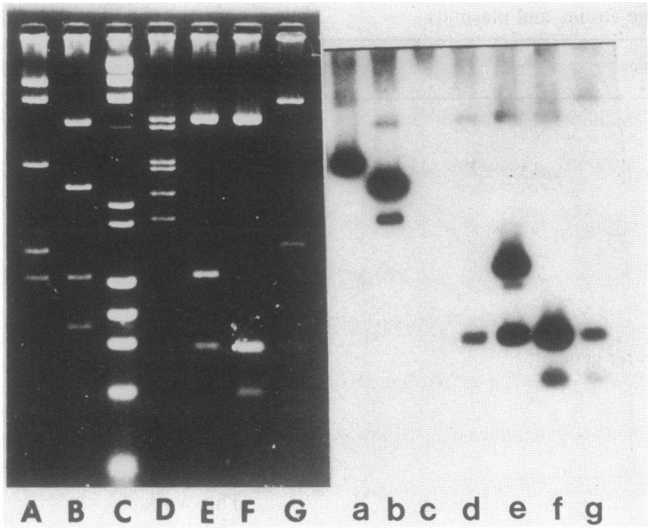

FIG. 1. Evidence that the *lit*(Con) mutations reside in the DNA element e14. Southern blot of an agarose gel of restriction endonuclease-digested DNAs of plasmids pAG2 and pHB101 with e14 DNA inserts. The blot was probed with the 2.3-kb fragment conferring the Lit(Con) phenotype. Lanes: A and B, pAG2 and pHB101, respectively, digested with *Hin*dIII-*Ava*I; C, lambda *Hin*dIII and φX174 *Hae*III molecular weight markers of 23.1, 9.4, 6.6, 4.4, 2.3, 2.0, 1.4, 1.1, 0.87, 0.60, and 0.31 kb; D and E, pAG2 and pAE1, respectively, digested with *Hin*dIII-*Eco*RV; F and G, pAE1 and pHB101, respectively, digested with *Eco*RV-*Bgl*II-*Hin*dIII. Lanes A to G, stained gel; lanes a to g, Southern blot. The second band from the top in lane f is a doublet.

and 20 μg of all 20 amino acids except methionine per ml. [^{35}S]methionine (Amersham) was added to 50 μCi/ml.

Labeled cells were lysed and fractionated by isopycnic centrifugation as described previously (27). The fractions were concentrated by trichloroacetic acid precipitation at a final volume of 5% trichloroacetic acid before electrophoresis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by the method of Laemmli (13) on 15% acrylamide gels at 100 V with constant cooling. Autoradiography was done with Kodak X-AR film.

Computer program. The Genepro program (version 4.00; Riverside Scientific) was used to analyze the DNA sequence, protein hydropathy, and potential protein secondary structure. The program uses the formula of Hoops and Woods for protein hydropathy analysis with a window of seven amino acids.

RESULTS

The *lit*(Con) mutations are in DNA element e14. There were three reasons to believe that the *lit*(Con) mutations may reside in the chromosomal element e14. First, both *lit*(Con) mutations and e14 map at 25 min on the *E. coli* chromosome (4, 11). Second, both the region of *lit*(Con) mutations and e14 seem to be common to only K-12 strains of *E. coli* and do not exist in B strains. Third, the loss of the Lit(Con) phenotype is usually associated with large deletions with at least one common endpoint, suggesting that the loss is due to the excision of a prophage or transposon (12).

To determine whether *lit*(Con) mutations are in e14, we performed Southern hybridizations with two plasmids (from Charles Hill) which contained the entire e14 element (4) in two *Hin*dIII fragments cloned from the excised circular element (see Fig. 2). Subclone pAG2 contains the large, approximately 8.5-kb, *Hin*dIII fragment in pBR313, and subclone pHB101 contains the two joined ends of e14 in the integrated state in pBR325. The *Hin*dIII-digested pAG2 and pHB101 DNAs were hybridized with the nick-translated 2.3-kb *Eco*RV fragment capable of conferring the Lit(Con) phenotype (Fig. 1). Both *Hin*dIII subclones showed strong hybridization, which is expected if *lit*(Con) mutations are in e14, because the 2.3-kb *Eco*RV fragment has a *Hin*dIII site and therefore would be divided between the two subclones. The Southern hybridizations also showed that *lit*(Con) mutations are close to the left-hand *Hin*dIII site of e14. The *Eco*RV fragment hybridized to the 3.5-kb *Ava*I-*Hin*dIII fragment of pAG2 and the 2.5-kb *Hin*dIII-*Ava*I fragment of

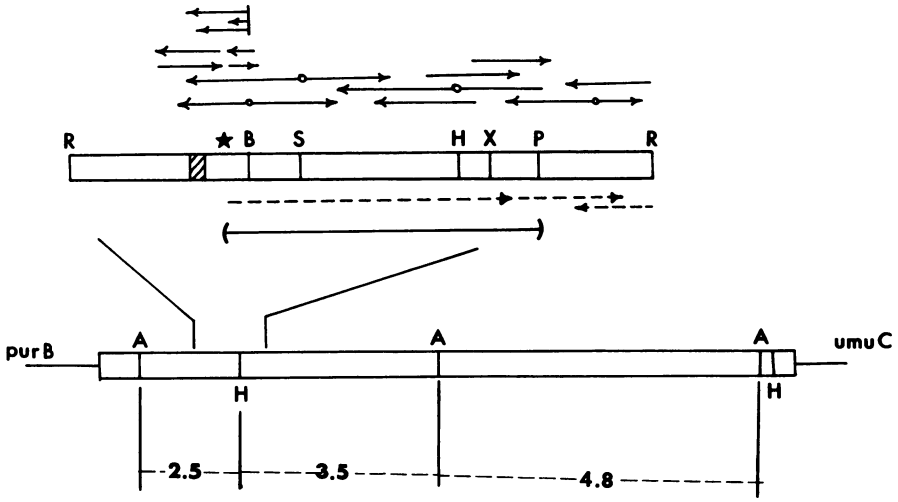

FIG. 2. Restriction map of e14 showing the location of the *lit* gene and the sequencing strategy. Symbols: ⟵⟶, maximum sequence required for the Lit(Con) activity (see text); ⟶, position and direction of the predicted ORFs; →, sequences determined by the dideoxy technique; ↦, sequences determined by the Maxam-Gilbert technique; ★, position of the C-to-T base pair change in the three *lit*(Con) mutants; ▨, position of the 8-base-pair repeats. Restriction sites: A, *Ava*I; B, *Bgl*II; H, *Hin*dIII; P, *Pvu*I; R, *Eco*RV; S, *Sca*I; X, *Xba*I. The approximate size of the e14 *Hin*dIII-*Ava*I restriction fragments, in kilobases, was estimated from Brody et al. (4).

pHB101 (Fig. 1, lanes a and b), which are adjacent to the left-hand *Hin*dIII site (Fig. 2). The 2.3-kb *Eco*RV insert also shares other restriction sites with the e14 subclones (Fig. 1, lanes D to G and d to g), consistent with the partial restriction map shown in Fig. 2.

Determining the DNA sequence of the *lit* region and the minimal sequence required for the Lit(Con) phenotype. The minimal DNA sequence required for the Lit(Con) phenotype was determined by a combination of site-specific mutagenesis, subcloning, and *Bal* 31 digestion of the 2.3-kb *Eco*RV fragment conferring the Lit(Con) phenotype. The plasmids with altered inserts were transformed into JM101 cured of e14, and the Lit(Con) phenotype was determined as described in Materials and Methods. Neither of the two fragments due to digesting the 2.3-kb *Eco*RV fragment with *Hin*dIII were able to confer the phenotype when subcloned into pBR328, suggesting that the *Hin*dIII site lies in a region required for the Lit(Con) phenotype. Also, mutations created by filling in the unique *Bgl*II or *Xba*I site inactivated the Lit(Con) phenotype, suggesting that these sites also lie in regions essential for activity. We also constructed *Bal* 31 deletions, ΔRI3 and ΔRI20, which removed about 700 and 800 base pairs of the left end of the 2.3-kb *Eco*RV fragment, respectively. The shorter deletion, ΔRI3, did not remove sequences required for activity, whereas the longer deletion, ΔRI20, left a fragment which could not confer the Lit(Con) phenotype. Therefore, the sequences required for Lit(Con) activity extend to somewhere between the endpoints of the two deletions. The unique *Pvu*I site apparently does not lie in required sequences, since a subclone of a fragment from the endpoint of deletion ΔRI3 to the *Pvu*I site conferred the Lit(Con) phenotype. We conclude that all of the sequences required for the Lit(Con) phenotype are contained in the region from slightly to the right of the ΔRI3 deletion endpoint to somewhere to the left of the *Pvu*I site. Incidentally, the *sfiC* gene on e14, which also contains a *Hin*dIII site (15), is probably on the right end of e14, because it is toxic in high-copy-number plasmids.

We sequenced the region from 347 base pairs leftward of the *Bgl*III site, designated nucleotide 1, to the rightward *Eco*RV site, designated nucleotide 1772, and the results are shown in Fig. 3. The sequence reveals two complete open reading frames (ORFs). The longer of these ORFs probably encodes a protein beginning with an ATG codon at nucleotide 272 and ending with a TGA codon at nucleotide 1164. This ORF could encode a polypeptide of 34 kDa. The second ORF could encode a protein beginning either at nucleotide 1125 with a GTG or at nucleotide 1228 with an ATG and extending to the TAG at nucleotide 1548. This frame could encode a polypeptide of about 17 kDa. We had previously reported (12) that the *lit*(Con) mutation *lit-6* caused the overproduction of two polypeptides of approximately 35 and 18 kDa in minicells. Because of their size, these two observed proteins are likely to be the products of ORF1 and ORF2, respectively.

We also sequenced the deletions, ΔRI3 and ΔRI20, to determine their endpoints (Fig. 3). Since the deletion ΔRI3, which deletes to 61 base pairs outside of ORF1, had no effect on the Lit(Con) phenotype, but the deletion ΔRI20, extending 29 base pairs into the coding sequence of ORF1, did abolish the phenotype, we think that the 34-kDa polypeptide is probably necessary for the Lit(Con) phenotype. On the other hand, the 17-kDa protein encoded by ORF2 is probably not required for the Lit(Con) phenotype, since a subclone that contains DNA from the ΔRI3 deletion endpoint to the *Pvu*I site within ORF2 still conferred the Lit(Con)

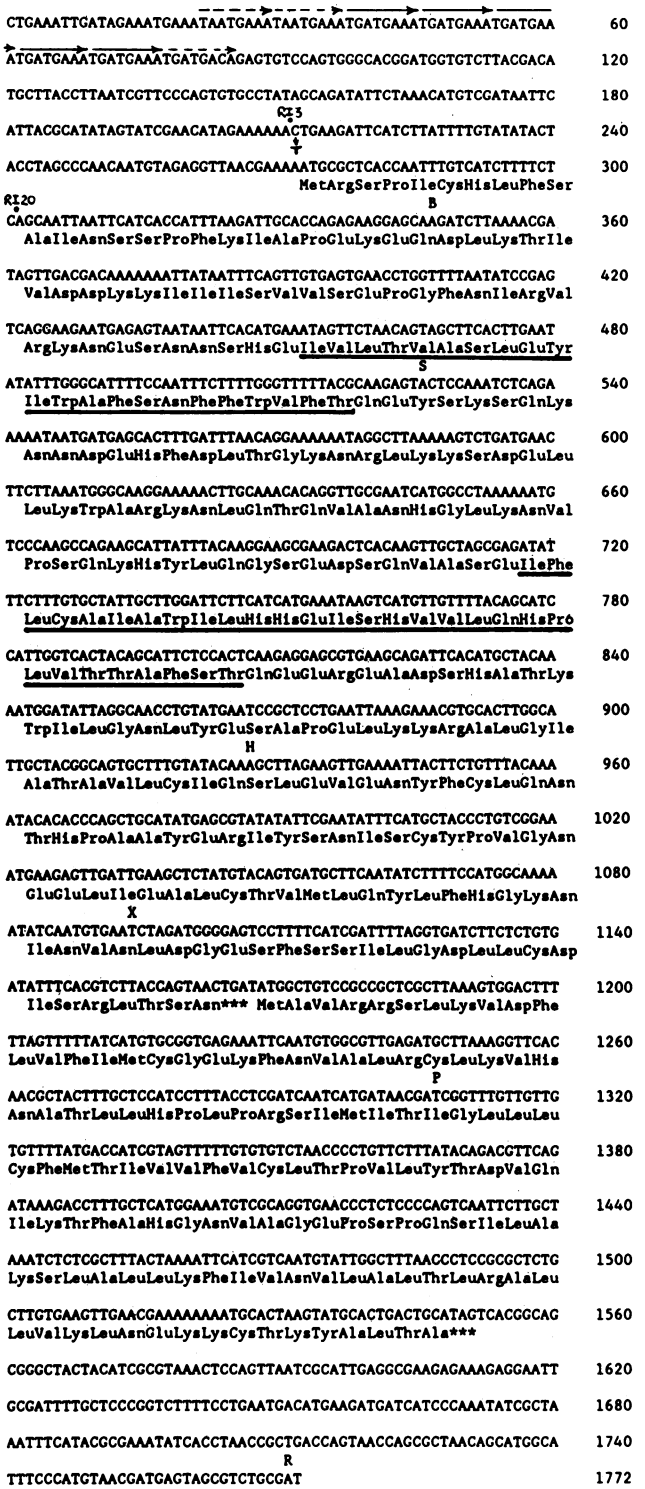

FIG. 3. Nucleotide sequence of the *lit* gene region and surrounding sequences. Capital letters above the sequence mark the positions of restriction sites: B, *Bgl*II; S, *Sca*I; H, *Hin*dIII; X, *Xba*I; P, *Pvu*I; R, *Eco*RV. The C-to-T base pair change of the *lit*(Con) mutations is shown at nucleotide 212. The two *Bal* 31 deletion endpoints, RI3 and RI20, are shown by dots at nucleotides 221 and 301. The perfect direct repeats are denoted by solid arrows, and the imperfect direct repeats are denoted by dashed arrows. The deduced amino acid sequence of the *lit* gene is also shown, with the two hydrophobic domains underlined.

phenotype. It is worth repeating that these experiments were performed with a strain cured of the e14 element to preclude complementation by the endogenous products of either ORF1 or ORF2. Since only ORF1 is required, we will subsequently refer to ORF1 as the *lit* gene and its product as gp*lit*.

There are other features of the sequence worth mentioning. The sequence of the *lit* gene is unusually rich in adenosines and thymidines, which together account for 60% of the total nucleosides. Also, upstream of the ORF1 there is an unusual set of tandem repeats composed of five contiguous perfect repeats of the 8-base-pair sequence TGAT GAAA plus a total of four imperfect repeats flanking the conserved repeats. Such repeats are often found near origins of replication (10, 14), but can be regulatory elements for certain genes (21). The repeats do not have a function related to expression of gp*lit*, at least in the plasmid clones, since the expression of the Lit(Con) phenotype is not affected by the deletion of the repeats. Although it is possible that the sequence plays some role in the expression of the *lit* gene in e14, we think it more likely that the sequence has some other role, perhaps vestigial, in the e14 element.

The *lit*(Con) mutations are probably up-promoter mutations. We performed a restriction-fragment-mixing experiment to try to localize the position of *lit*(Con) mutations. Since *lit*(Con) mutations cause the overproduction of the 34-kDa protein, it seemed likely that they would be upstream of ORF1. We could distinguish clones of the wild-type DNA from clones with a *lit*(Con) mutation, since more gp*lit* is required to show the Lit(Con) phenotype at higher temperatures and only the mutant clones show the phenotype at 42°C. Plasmid pAE1, with the wild-type insert, and plasmids pM6E and pM21E, with the *lit*(Con) mutations *lit-6* and *lit-21*, respectively, were cut with *Bgl*II and *Eco*RI. The 1.6- and 4.9-kb fragments from each plasmid were isolated and religated in mix-and-match combinations. The source of the 1.6-kb fragment determined the phenotype of the religated plasmid. Only if the 1.6-kb fragment was from a plasmid with a lit(Con) mutation was the phenotype Lit(Con). We conclude that the *lit*(Con) mutations are upstream of the *Bgl*II site in ORF1, since this is the region of insert DNA included in the 1.6-kb fragments.

We sequenced the DNA upstream of the *Bgl*II site in DNA from three independently isolated mutants containing the *lit*(Con) mutations *lit-6*, *lit-7*, and *lit-21*. All have the same CG-to-TA transition at 60 nucleotides upstream of the start codon in ORF1 (Fig. 3). This region can be seen to bear considerable resemblance to the *E. coli* promoter consensus sequence, and the mutations increase the resemblance to the consensus promoter (Fig. 4). Because of this resemblance, we thought that *lit*(Con) mutations could be up-promoter mutations. If so, they should increase the transcription of the *lit* gene.

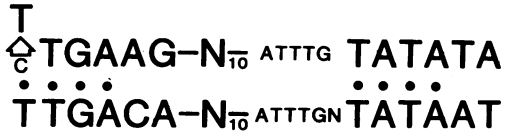

FIG. 4. The *lit*(Con) mutations increase the resemblance of the putative *lit* promoter to the *E. coli* consensus promoter sequence. The upper line contains the sequence of the *lit* promoter, and the bottom line contains the consensus promoter. The *lit* mutations are all the same C-to-T transition, and their position is denoted by the arrow. The best-conserved nucleotides of the −35 box and the Pribnow box are noted by dots.

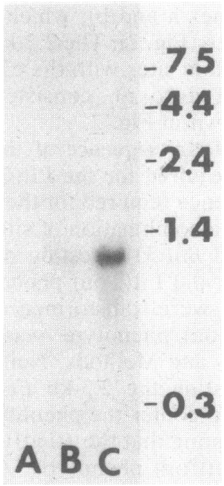

FIG. 5. The *lit*(Con) mutations increase the transcription of the *lit* gene. Total RNAs were purified from JM101 *lit*^{0} (lane A), JM101 *lit*$^+$ (lane B), and JM101 *lit-6* (lane C), processed as described in Materials and Methods, and probed with a *Bgl*II-*Hin*dIII fragment internal to the *lit* gene. The numbers denote the size of the RNA ladder in kilobases.

From the Northern (RNA) hybridization experiment shown in Fig. 5, it is apparent that *lit*(Con) mutations do increase the transcription of the *lit* gene. In this experiment, we isolated the total RNA from three different *E. coli* strains, one of which had a *lit*(Con) mutation in e14, one which had wild-type e14, and one of which was cured of e14. After electrophoresis, the RNAs were blotted and probed with the *Bgl*II-*Hin*dIII fragment internal to the *lit* gene (Fig. 5). RNA from both the cells containing wild-type e14 and the cells in which the e14 had a *lit*(Con) mutation showed hybridization to only one RNA of about 1.1 kb in the original autoradiograph, and this RNA was missing in the cells cured of e14. Therefore, it is probably the RNA transcribed from the *lit* gene. From the density of the band, we estimate that the *lit*(Con) mutation increases the amount of this RNA about 10-fold. This evidence strongly supports the conclusion that *lit*(Con) mutations are up-promoter mutations. Incidentially, the observation that some of the RNA is made from wild-type e14 is consistent with other work, which showed that some Lit protein is present in cells harboring wild-type e14 (Bergsland and Snyder, submitted). This RNA is probably long enough to encode only the 34-kDa Lit protein of ORF1, but not the 17-kD product of ORF2 also. Since we thought that *lit*(Con) mutations cause the overproduction of both proteins, it seems possible that this RNA is processed, although then we might expect to see some of the longer, unprocessed precursor.

Cellular location of the Lit protein. Transmembrane proteins often have characteristic hydrophobic amino acid domains. A hydropathy plot of the predicted Lit protein sequence revealed two hydrophobic stretches that could traverse the membrane (Fig. 3). The first stretch, of 22 amino acids, is encoded by the DNA from nucleotides 452 to 518. The longer second stretch, of 30 amino acids, is encoded by nucleotides 716 to 806. These two domains have overall hydrophobic properties, although they do contain some charged amino acids. The presence of such hydrophobic sequences suggested that gp*lit* might be a membrane protein, and so we examined the location of the *lit* gene product in the cell.

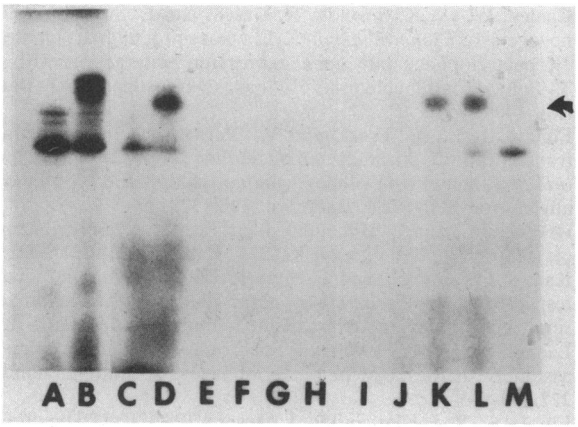

FIG. 6. The *lit* protein is enriched in the inner membrane preparations from *E. coli*. The plasmid-encoded proteins were labeled in maxicells, and the cells were fractionated into periplasm, cytoplasm, and membrane components as described in Materials and Methods. A portion of each membrane pellet was then banded in an isopycnic sucrose gradient. Lanes: A, total proteins from the pABglII+4 plasmid which has a frameshift mutation in the *lit* gene; B, total proteins from the pM6E1 plasmid containing the *lit* gene; C and D, total membrane pellet proteins from pABglII+4 and pM6E1 plasmids, respectively; E, to G, outer membrane fractions from the isopycnic gradient of the pM6E1 cells; H to J, mixed membrane fractions from the gradient; K to M, inner membrane fractions from the gradient. The gp*lit* is the diffused band marked by the arrow at the top of the gel.

We labeled the plasmid-encoded proteins by the maxicell technique and then fractionated the cell into cytoplasmic, periplasmic, and membrane components. A portion of the membrane fraction was further separated into outer and inner membrane fractions with isopycnic sucrose gradients. There were usually five distinct bands due to the plasmid-encoded proteins: the two forms of β-lactamase, chloramphenicol acetyltransferase, and the two insert-encoded polypeptides of 34 and 17 kDa. The 34-kDa polypeptide is the *lit* gene product identified previously by three criteria: (i) it is the correct size, (ii) it gives the same diffused band as the 34-kDa protein overproduced in the minicell experiments (12), and (iii) it is missing in cells containing the pABglII+4 plasmid that has a frameshift mutation at the *Bgl*II site in ORF1 (Table 1). When the maxicell-labeled proteins (Fig. 6) were fractionated into the cytosol, periplasm, and inner and outer membranes, the antibiotic resistance proteins segregated as expected. The chloramphenicol acetyltransferase was enriched in the cytosolic fraction, and the mature and precursor forms of β-lactamase polypeptides were enriched in the periplasm and the cytoplasm, respectively (data not shown). The only detectable membrane protein encoded by the plasmid vector is the tetracycline resistance protein, which is 40 kDa. However, in these *Eco*RV clones the *tet* gene is disrupted and the protein should not be produced. The *omp* proteins segregated with the outer membrane, as apparent from the stained-gel pattern (data not shown).

In contrast to the other proteins, gp*lit* was enriched in the inner membrane fraction. The Lit polypeptide was enriched in the total membrane fraction in the pM6E1-containing cells (Fig. 6, lane D), and when a portion of the membrane pellet was banded on an isopycnic sucrose gradient, a diffused band of the same molecular mass as gp*lit* further segregated into the inner membrane fractions (Fig. 6, lanes K to M). Some of this band also appeared in the mixed-membrane fraction (lanes H to J), but is largely absent on the outer membrane fractions (lanes E to G). The lower band present in lanes L and M probably represents some of the chloramphenicol acetyltransferase which was trapped in the total membrane pellet.

DISCUSSION

We have shown that the Lit(Con) phenotype for blocking gene expression late in infection by bacteriophage T4 is due to the overproduction of a 34-kDa protein, gp*lit*, encoded by the cryptic DNA element e14. The *lit* gene straddles the *Hin*dIII site on the left side of e14 closest to *purB* (4) and is transcribed from left to right with respect to the integrated e14 map. We have also sequenced the *lit* gene and determined the base pair changes due to three *lit*(Con) mutations, all three of which were CG-to-TA transitions at the same base pair, 60 nucleotides upstream of the presumed start codon of gp*lit*. These mutations result in a promoter which more closely approximates the consensus promoter in *E. coli* and cause an increase in the amount of the *lit* gene RNA by Northern analysis. We also present evidence that gp*lit* is an inner membrane protein, since it has the characteristic hydrophobic domains and segregates with the inner membrane during cell fractionation.

All of our evidence thus far supports the idea that the *lit* gene is unregulated and normally expressed at low levels from a weak promoter just upstream of the gene. It is of interest why e14 expresses this gene in the dormant state, but this could be difficult to determine, since all that is known about e14 is that it can excise and reintegrate and that it does not contain genes essential to *E. coli* (4, 11, 15). It seems likely that e14 is a defective prophage or integrated plasmid, and many of its functions could be vestigial. The Lit protein, even when grossly overproduced, has no apparent effect on the *E. coli* host. It is only after infection by bacteriophage T4 that its presence becomes known.

The mechanism through which gp*lit* blocks gene expression late in T4 infection still remains a mystery. Its effect becomes apparent when the late T4 gene 23 is first transcribed and translated. The interaction between gp*lit* and a short sequence within T4 gene 23, the *gol* site, blocks the expression of gene 23 in *cis* (6; Bergsland and Snyder, submitted) and other genes in *trans* (7; C. Kao and L. R. Snyder, to be published). Considering our evidence that gp*lit* is an inner membrane protein, this interaction presumably occurs on the inner surface of the inner membrane.

The product of T4 gene 23 is the major capsid protein gp23, which is thought to bind to the T4-encoded inner membrane protein gp20 during the normal assembly of the procapsid (3). How gp23 is targeted to the membrane is unknown. From other work (Bergsland and Snyder, submitted), we know that the Lit protein seems to cause the expression of gene 23 to terminate in the *gol* region. Perhaps the *gol* site is the sequence that targets gp23 to an assembly complex which includes T4 gp20 in the membrane. According to this hypothesis, translation (and/or transcription) normally pauses at the *gol* site until the site is properly docked at the membrane assembly complex. Then, and only then, can translation (or transcription) continue. When gp*lit* is overproduced, it somehow interferes with the docking event, and the translation or transcription of gp23 is inhibited, accounting for the *cis* effect of gp*lit* on the expression of gene 23. Also, according to this model, T4 *gol* mutations change the sequence of the *gol* site so that it no longer interacts as strongly with gp*lit*, thus averting the more permanent block of gp23 synthesis. This interpretation is

consistent with all our evidence so far on the *gol*-gp*lit* interaction (6, 7, 12; Bergsland and Snyder, submitted). Much more difficult to explain, however, is how this interaction causes the observed *trans* inhibition of the expression of other genes. Perhaps the arrested gene 23 transcriptional-translational complexes somehow induce a *trans* inhibitor of all gene expression. The normal function of this *trans* inhibition may be to reduce the expression of other T4 late genes and therefore delay the progress of the infection, if T4 heads are not being assembled properly.

There are other known situations in which gene products encoded by two different phages or by a phage and a plasmid interact to cause a general effect on gene expression and/or a general shutdown of cellular functions (2, 19, 28). In at least one such instance, the *rexA* and *rexB* genes of phage lambda may encode membrane proteins (5). The *rex* genes of λ are also expressed in the lysogenic state, and although they normally inhibit only *r*II mutants of T4, they will even inhibit wild-type T4 if overproduced (24). Because of their similarities, it seems possible that gp*lit* of e14 and other proteins such as the *rex*A and/or *rex*B gene products of λ have a common function.

ACKNOWLEDGMENTS

We thank Kristin Bergsland for helpful discussions, Charles Hill for generously providing the e14 subclones, Jerry Dodgson and members of his laboratory for materials and advice in Maxam-Gilbert sequencing, and Robert Brubaker and members of his laboratory for advice on separating membrane fractions with isopycnic sucrose gradients.

C.K. acknowledges support from a University Cellular and Molecular Biology assistantship and the Rosenberg fellowship. This work was supported by Public Health Service grant GM-28001 from the National Institutes of Health and by National Science Foundation grant DM8617142.

LITERATURE CITED

1. **Aiba, H., S. Adhya, and B. deCrombrugge.** 1981. Evidence for two functional gal promoters in intact *Escherichia coli*. J. Biol. Chem. **256**:11905–11910.
2. **Benzer, S.** 1955. Fine structure of a genetic region in bacteriophage. Proc. Natl. Acad. Sci. USA **41**:344–354.
3. **Black, L., and M. Showe.** 1983. Morphogenesis of the T4 head, p. 219–245. *In* C. K. Mathews, E. M. Kutter, G. Mosig, and P. B. Berget (ed.), Bacteriophage T4. American Society for Microbiology, Washington, D.C.
4. **Brody, H., A. Greener, and C. Hill.** 1985. Excision and reintegration of the *Escherichia coli* chromosomal element e14. J. Bacteriol. **161**:1112–1117.
5. **Campbell, J. H., and B. G. Rolfe.** 1975. Evidence for a dual control of the initiation of host-cell lysis caused by phage lambda. Mol. Gen. Genet. **155**:1–8.
6. **Champness, W., and L. Snyder.** 1982. The *gol* site: a cis-acting bacteriophage T4 regulatory region that can affect expression of all the T4 late genes. J. Mol. Biol. **155**:395–407.
7. **Champness, W., and L. Snyder.** 1984. Bacteriophage T4 *gol* site: sequence analysis and effects of the site on plasmid transformation. J. Virol. **50**:555–562.
8. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. USA **69**:2110–2114.
9. **Cooley, W. C., K. Sirotkin, R. Green, and L. Snyder.** 1979. A new gene of *Escherichia coli* K-12 whose product participates in T4 bacteriophage late gene expression: interaction with the T4-induced polynucleotide 5′-kinase 3′-phosphatase. J. Bacteriol. **140**:83–91.
10. **Finlay, B. L., L. Frost, and W. Parachych.** 1986. Origin of transfer of IncF plasmids and nucleotide sequence of the type II *oriT*, *traM*, and *traY* alleles from colB4-K98 and type IV *traY* alleles from R100-1. J. Bacteriol. **168**:132–139.
11. **Greener, A., and C. Hill.** 1980. Identification of a novel genetic element in *Escherichia coli* K-12. J. Bacteriol. **144**:312–321.
12. **Kao, C., E. Gumbs, and L. Snyder.** 1987. Cloning and characterization of the *Escherichia coli lit* gene, which blocks bacteriophage T4 late gene expression. J. Bacteriol. **169**:1232–1238.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Lin, L. S., Y. J. Kim, and R. J. Meyers.** 1987. The 20 bp directly repeated DNA sequence of broad host range plasmid R1162 exerts incompatibility in vivo and inhibits R1162 DNA replication in vitro. Mol. Gen. Genet. **208**:390–397.
15. **Maguin, E., H. Brody, C. Hill, and R. D'Ari.** 1986. SOS-associated division inhibition gene *sfiC* is part of an excisable element e14 in *Escherichia coli*. J. Bacteriol. **168**:464–466.
16. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance in *Escherichia coli*. J. Bacteriol. **145**: 1110–1112.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Maxam, A., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. USA **74**:560–564.
19. **Miller, J., and M. Malamy.** 1984. Regulation of the F-factor *pif* operon: *pifO*, a site required in *cis* for autoregulation, titrates the *pifC* product in *trans*. J. Bacteriol. **160**:192–198.
20. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Miller, V. L., R. K. Taylor, and J. Mekalanos.** 1987. Cholera toxin transcriptional activator *tox*R is a transmembrane DNA binding protein. Cell **48**:271–279.
22. **Sancar, A., R. Wharton, S. Seltzer, G. Kacinski, N. Clarke, and W. D. Rupp.** 1981. Identification of the *uvr*A gene product. J. Mol. Biol. **148**:45–62.
23. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
24. **Shinedling, S., D. Parma, and L. Gold.** 1987. Wildtype bacteriophage T4 is restricted by the lambda *rex* genes. J. Virol. **61**:3790–3794.
25. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
26. **Stuart, P., M. Ito, C. Stewart, and S. Conrad.** 1985. Induction of cellular thymidine kinase occurs at the mRNA level. Mol. Cell. Biol. **5**:1490–1497.
27. **Straley, S. C., and R. R. Brubaker.** 1981. Cytoplasmic and membrane proteins of yersiniae cultivated under conditions simulating intracellular environment. Proc. Natl. Acad. Sci. USA **78**:1224–1228.
28. **Toothman, P., and I. Herskowitz.** 1979. Rex-dependent exclusion of lambdoid phages. I. Prophage requirement for exclusion. Virology **102**:133–146.
29. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.